

## Agro-Morphological Traits Variability in Nepalese Soybean Landraces

Pradip Thapa\*<sup>1</sup>, Ram Prasad Mainali<sup>2</sup>, Ajaya Karkee<sup>3</sup>, Krishna Hari Ghimire<sup>4</sup>, Bal Krishna Joshi<sup>5</sup>

<sup>1</sup>National Agriculture Genetic Resources Centre, NARC, Khumaltar, Lalitpur, Nepal  
Email: [pradip.thapa876@gmail.com](mailto:pradip.thapa876@gmail.com) | ORCID: <https://orcid.org/0000-0002-2080-6517>

<sup>2</sup>National Agriculture Genetic Resources Centre, NARC, Khumaltar, Lalitpur, Nepal  
Email: [mainalism.rp@gmail.com](mailto:mainalism.rp@gmail.com) | ORCID: <https://orcid.org/0000-0002-4637-3676>

<sup>3</sup>National Agriculture Genetic Resources Centre, NARC, Khumaltar, Lalitpur, Nepal  
Email: [ajayakarkee@gmail.com](mailto:ajayakarkee@gmail.com) | ORCID: <https://orcid.org/0000-0002-5074-7079>

<sup>4</sup>National Plant Breeding and Genetics Research Centre, NARC, Khumaltar, Lalitpur, Nepal  
Email: [ghimirekh@gmail.com](mailto:ghimirekh@gmail.com) | ORCID: <https://orcid.org/0000-0002-3393-290X>

<sup>5</sup>National Agriculture Genetic Resources Centre, NARC, Khumaltar, Lalitpur, Nepal  
Email: [joshibalak@yahoo.com](mailto:joshibalak@yahoo.com) | ORCID: <https://orcid.org/0000-0002-7848-5824>

\*Corresponding author

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### Abstract

A comprehensive and detailed characterization of genetic resources is crucial for both harnessing specific traits and protecting unique germplasm. The research was conducted at the plant breeding research field of the National Genebank, Nepal in 2021. This study aimed to evaluate the variability among soybean landraces from various regions of the country by analyzing agro-morphological traits. 77 soybean landraces, sourced from 24 districts across the nation were characterized by following augmented block design. 11 qualitative and twelve quantitative characters were recorded following the descriptor developed by Bioversity International. Descriptive analysis was done by using MS Excel 2019 and multivariate analysis of quantitative characters was done with OriginPro 2024 software and R Studio 3.5 version. Among the studied quantitative characters, yield (112.71%), 1000 grain wt. (38.45%) and plant height (27.28%) showed high variability among the landraces. A dendrogram was generated for the 77 soybean landraces utilizing the UPGMA clustering approach, which incorporates average linkage and Euclidean distance, to analyze 12 quantitative traits. The cluster segmentation organized the landraces into two distinct categories with a distance of 111.42. The soybean landraces studied showed considerable variability in various qualitative and quantitative traits. This observed diversity offers valuable potential for both breeding programs and direct applications. These promising landraces need further screening through multi-environment trials and the application of various biotechnological tools.

### Keywords

Clustering; Dendrogram; Landrace; Soy

## 1. Introduction

Soybean (*Glycine max* (L.) Merr.) is a leguminous plant species that originated from Eastern Asia (Nwokolo, 1996; Singh *et al.*, 2007), distinguished as a multipurpose and remarkable field crop of the modern era (Mishra *et al.*, 2024). Its numerous benefits extend beyond improving soil health to supporting various industries and the medicinal sector globally (Figueredo, 2014). In 2022,

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a total of 129,523,964 hectares of land was used for soybean cultivation worldwide, resulting in a production of 348,856,427 tons, with Brazil leading as the largest producer at 154.6 million tons, followed by the United States (FAO, 2023). In Brazil, over 90% of this area is planted with genetically modified (GM) varieties, accounting for about 95% of total production (Gbadegesin *et al.*, 2022). Following lentils, soybean ranks as Nepal's second most significant grain legume (Dhakal, 2020). In Nepal, soybean cultivation spans 23,030 hectares, yielding 30,648 tons, with a productivity rate of 1.33 tons per hectare (MoALD, 2022/23). It can adapt to a range of agroecological zones, thriving at altitudes between 200 and 2000 meters above sea level (Darai *et al.*, 2020). Mid hills regions of Nepal contribute 80% of the country's soybean area and production (Krunpik *et al.*, 2021). However, high demand for soymeal in the poultry business, soybeans are becoming more and more popular as stand-alone crops in the foothills and inner Terai areas of Nepal (Pokhrel *et al.*, 2014).

Because of its distinct chemical makeup and wide range of uses in food, feed, and industrial products (Shea *et al.*, 2020), soybeans are now regarded as one of the most valuable and affordable agricultural commodities (Thapa *et al.*, 2019). Of all the edible legumes, soybeans have the second-highest oil content and the greatest protein content among cereal and legume species (Liu, 2012; Miah *et al.*, 2024). Approximately 40% premium protein, 6% mineral, 29% carbs, 25% essential oil and 85% major lipids are found in soybeans (Aditya *et al.*, 2019). Soy protein has been recognized as a beneficial food for human health including reducing cholesterol and lowering the chance of cardiovascular disease, cancer, osteoporosis and other chronic conditions (Bhartiya *et al.*, 2020). Soybean isoflavones, particularly genistein and daidzein, have been shown to help prevent and treat osteoporosis as well as reduce the incidence of several malignancies (Kim, 2021). When added to a variety of culinary applications, soy hulls which have a dietary fibre content of about 65% make an excellent source of fiber (Kalaiselvan *et al.*, 2010).

Soybean productivity in Nepal is significantly lower compared to neighbouring countries (Paudel *et al.*, 2015; Thapa *et al.*, 2019). Key factors contributing to this low yield include poor crop management, disease prevalence, and a lack of diverse varieties suited for both intercrop and sole cropping systems (Kaini, 2018; Paudel *et al.*, 2015). Germplasm possesses valuable traits, including a high concentration of advantageous components, resistance to biotic stress, and resilience to changing climate (Kim *et al.*, 2022). To support this effort, gene banks are diligently working to secure and preserve the diversity of genetic resources. China and Korea hold the largest collections of soybean germplasm, with 26,000 accessions preserved in each country (Kefale and Wang, 2022). Currently, one hundred fifty-five soybean germplasm collected from various regions across the country is preserved in long-term storage at Nepal's national gene bank (Genebank, 2022).

Landraces are valuable sources of genetic variability and assessing the genetic variation and relationships among conserved germplasm is crucial (Kyratzis *et al.*, 2019). The Nepalese soybean landraces exhibit a significant morphological variation in terms of maturity duration, bloom colour, plant growth, pod and seed count per plant and economic production (Darai *et al.*, 2020). Despite this wide diversity, it has yet to be effectively incorporated into the soybean improvement scheme in Nepal (Joshi, 2017). Assessing and documenting landraces are crucial for accurately recording and pinpointing materials with

potential value for targeted breeding objectives (Lazaridi *et al.*, 2024). A comprehensive and detailed characterization of genetic resources is crucial for both harnessing specific traits and protecting unique germplasm (Prasanna, 2012). Evaluating genetic variation in plant material can be achieved through phenotypic assessment, which assists plant breeders in choosing accessions for hybridization programs (Ghafoor *et al.*, 2002). Nepalese soybean landraces exhibit substantial genetic diversity, which is reflected in their agro-morphological traits. This study aimed to evaluate the variability among soybean landraces from various regions of the country by analyzing agro-morphological traits.

Materials and Methods

Research Materials and Design

The research was conducted at the plant breeding research field of the national gene bank, Nepal in 2021. The location is positioned at an elevation of 1368 meters, with 27°40' north-south and 85°20' east-west position. The plots used for the experiment are located on soil with a black loamy composition. Meteorological data for the soybean growing season of 2021 is depicted in Figure 1. Seventy-seven soybean landraces, sourced from 24 districts across the nation as shown in table 1, were planted in the experimental field for agro-morphological assessment using an augmented block design. Planting took place on 10<sup>th</sup> May 2021, during the main cropping season, following recommended fertilizer and management practices. The gross plot size was 6m<sup>2</sup> (3 m x 2 m), and each plot accommodated four rows of 3m length with a distance of 50cm×15cm between rows and plants respectively. Among the four rows, two middle rows were designated as sampling areas.

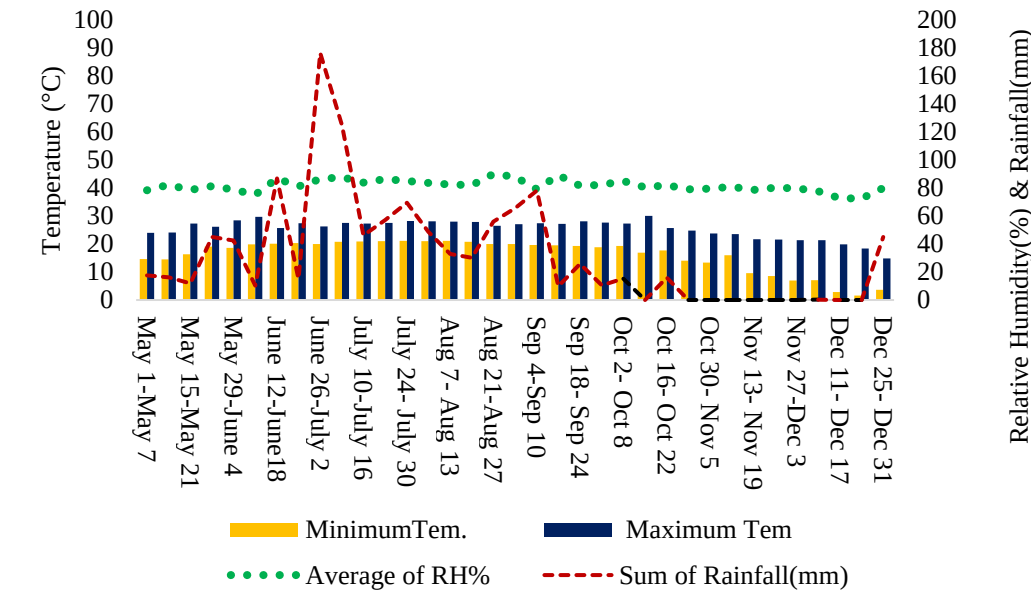


Figure 1. Average maximum and minimum temperatures (°C), total precipitation (mm), and average relative humidity (%) measured in Khumaltar, Lalitpur, Nepal, between May 2021 and Dec 2021 (*Source: National Agronomy Research Centre, Khumaltar, 2021*)

Table 1: Soybean Landraces and Their Collected Locations

| <i>S.N.</i> | <i>Landraces</i> | <i>Collected site</i> | <i>Local Name</i>  | <i>Altitude (m)</i> | <i>Latitude (N)</i> | <i>Longitude (E)</i> |
|-------------|------------------|-----------------------|--------------------|---------------------|---------------------|----------------------|
| 1           | C00702           | Bidur, Nuwakot        | Kalo bhatta        | 545                 | 27.051              | 85.007               |
| 2           | C00709           | Balche, Nuwakot       | Local bhatta       | 545                 | 27.051              | 85.007               |
| 3           | C00712           | Lahuti, Darchula      | Kalo bhatta        | 1619                | 29.543              | 80.345               |
| 4           | C00715           | Lahuti, Darchula      | Kalo bhatta        | 1619                | 29.543              | 80.345               |
| 5           | C00721           | Kheti, Darchula       | Kalo bhatta        | 2204                | 29.481              | 80.391               |
| 6           | C00723           | Kheti, Darchula       | Kalo bhatta        | 2204                | 29.481              | 80.391               |
| 7           | C00726           | Chilime, Rasuwa       | Local bhatta       | 2306                | 28.112              | 85.185               |
| 8           | C00730           | Golijung, Rasuwa      | Local bhatta       | 1947                | 28.010              | 85.018               |
| 9           | C00741           | Laharemane, Dolakha   | Mahilo Bhatmaas    | 1536                | 27.492              | 85.131               |
| 10          | C01363           | Sikhre, Sindhupalchok | Kalo bhatmaas      | 2011                | 27.041              | 85.053               |
| 11          | C01366           | Ghunsa, Sindhupalchok | Kalo bhatmaas      | 1675                | 27.055              | 85.040               |
| 12          | C02808           | Those, Ramechhap      | Khairo Bhatmaas    | 1857                | 27.360              | 86.172               |
| 13          | C02811           | Those, Ramechhap      | Seto Bhatmaas      | 1742                | 27.357              | 86.158               |
| 14          | C02812           | Meckchan, Ramechhap   | Kailo Bhatmaas     | 1739                | 27.357              | 86.589               |
| 15          | C02818           | Garjang, Ramechhap    | Khairo Bhatmaas    | 1973                | 27.389              | 86.192               |
| 16          | C02819           | Garjang, Ramechhap    | Seto Bhatmaas      | 1942                | 27.389              | 86.192               |
| 17          | C02824           | Mangaltar, Kavre      | Kalo bhatmaas      | 1536                | 27.492              | 86.431               |
| 18          | C02826           | Mangaltar, Kavre      | Kalo bhatmaas      | 1536                | 27.492              | 86.431               |
| 19          | C02829           | Pokharichaur, Kavre   | Kailo Bhatmaas     | 1461                | 27.340              | 85.482               |
| 20          | C02830           | Pokharichaur, Kavre   | Seto bhatmaas      | 1461                | 27.340              | 85.482               |
| 21          | C02831           | Pokharichaur, Kavre   | Kaili Bhatmaas     | 1450                | 27.034              | 85.481               |
| 22          | C02832           | Pokharichaur, Kavre   | Seti Bhatmaas      | 1450                | 27.343              | 85.481               |
| 23          | C02834           | Dhunge, Dolakha       | Seto Bhatmaas      | 2030                | 27.386              | 86.137               |
| 24          | C02837           | Dhunge, Dolakha       | Koilo Bhatmaas     | 2030                | 27.386              | 86.137               |
| 25          | C02838           | Jiri,Dolakha          | kalo Bhatti        | 2030                | 27.386              | 86.137               |
| 26          | C02839           | Sakhalpur, Dolakha    | Local Bhatmaas     | 1603                | 27.415              | 86.064               |
| 27          | C02842           | Oligaun, Dolakha      | Kalo bhatmaas      | 1570                | 27.483              | 86.064               |
| 28          | C03302           | Sindhupalchok         | Local Bhatta       | 1835                | 27.542              | 85.451               |
| 29          | C03613           | Dhunge, Dolakha       | Local bhatmaas     | 2030                | 27.386              | 86.137               |
| 30          | C03748           | Rukum                 | Local bhatmaas     | 1833                | 28.403              | 82.302               |
| 31          | C03749           | Khalanga, Jajarkot    | Local bhatmaas     | 2120                | 28.463              | 82.113               |
| 32          | C03751           | Khalanga,Jajarkot     | Local bhatmaas     | 2120                | 28.463              | 82.113               |
| 33          | C04784           | Dillichaur,Jumla      | Muse bhatmaas      | 2609                | 29.308              | 82.303               |
| 34          | C04980           | Nuwakot               | Local bhatmaas     | 741                 | 27.545              | 85.091               |
| 35          | C010066          | Triyuga,Udayapur      | Seto bhatmaas      | 142                 | 26.788              | 86.707               |
| 36          | C010627          | Kawasoti, Nawalparasi | Khairo bhatmaas    | 221                 | 27.390              | 84.080               |
| 37          | C010771          | Bagmati, Lalitpur     | Seto bhatmaas      | 530                 | 27.422              | 85.376               |
| 38          | C010795          | Mahakal,Lalitpur      | Isthaniya,bhatmaas | 1013                | 27.443              | 85.404               |
| 39          | C010853          | Gokulganga,Ramechhap  | Seto bhatmaas      | 1829                | 27.593              | 86.261               |
| 40          | C010860          | Gokulganga,Ramechhap  | Kailo bhatmaas     | 2011                | 27.588              | 86.262               |
| 41          | C010861          | Gokulganga,Ramechhap  | Kause bhatmaas     | 2011                | 27.588              | 86.262               |
| 42          | C010877          | Gokulganga,Ramechhap  | Kalo bhatmaas      | 1350                | 27.590              | 86.260               |
| 43          | C010922          | Gokulganga,Ramechhap  | Seto bhatmaas      | 1665                | 27.564              | 86.193               |
| 44          | C010928          | Gokulganga,Ramechhap  | Kause bhatmaas     | 1665                | 27.56               | 86.193               |
| 45          | C010931          | Gokulganga,Ramechhap  | Rato bhatmaas      | 1665                | 27.564              | 86.193               |

| <i>S.N.</i> | <i>Landraces</i> | <i>Collected site</i> | <i>Local Name</i> | <i>Altitude (m)</i> | <i>Latitude (N)</i> | <i>Longitude (E)</i> |
|-------------|------------------|-----------------------|-------------------|---------------------|---------------------|----------------------|
| 46          | C011090          | Mangala, Myagdi       | Local bhatta      | 1878                | 28.408              | 83.455               |
| 47          | C011113          | Mangala, Myagdi       | Kalo bhatmaas     | 1849                | 28.406              | 83.453               |
| 48          | C011131          | Mangala, Myagdi       | Nepali bhatta     | 1209                | 28.391              | 83.463               |
| 49          | C011146          | Mangala, Myagdi       | Seto bhatmaas     | 2119                | 28.415              | 83.447               |
| 50          | C011150          | Mangala, Myagdi       | Nepali bhatta     | 1780                | 28.415              | 83.439               |
| 51          | C011165          | Mangala, Myagdi       | Kalo bhatta       | 1780                | 28.415              | 83.439               |
| 52          | C0 11172         | Mangala, Myagdi       | Seto bhatmaas     | 1603                | 28.403              | 83.459               |
| 53          | C011174          | Mangala, Myagdi       | Kailo bhatmaas    | 1603                | 28.403              | 83.459               |
| 54          | C011855          | Meghang, Nuwakot      | Local bhatta      | 1294                | 27.961              | 85.110               |
| 55          | C011856          | Uttargaya, Rasuwa     | Seto bhatmaas     | 1152                | 27.950              | 85.116               |
| 56          | C011863          | Thaha, Makawanpur     | Seto bhatmaas     | 1692                | 27.654              | 85.118               |
| 57          | C011866          | Thaha, Makawanpur     | Seto bhatmaas     | 1701                | 27.644              | 85.118               |
| 58          | C011867          | Thaha, Makawanpur     | Seto bhatmaas     | 1701                | 27.644              | 85.118               |
| 59          | C011870          | Thaha, Makawanpur     | Seto bhatmaas     | 1701                | 27.644              | 85.118               |
| 60          | C011952          | Chhayanth, Mugu       | Seto bhatmaas     | 2400                | 29.320              | 82.092               |
| 61          | C011979          | Tuwachung, Khotang    | Seto bhatmaas     | 431                 | 27.175              | 86.474               |
| 62          | C011994          | Diktel, Khotang       | Seto bhatmaas     | 1641                | 27.221              | 86.787               |
| 63          | C012073          | Diktel, Khotang       | Seto bhatmaas     | 1523                | 27.215              | 86.795               |
| 64          | C012096          | Diktel, Khotang       | Nepale bhatmaas   | 1603                | 27.217              | 86.792               |
| 65          | C012115          | Tuwachung, Khotang    | Seto bhatmaas     | 649                 | 27.095              | 86.373               |
| 66          | C012116          | Tuwachung, Khotang    | Khairo bhatmaas   | 649                 | 27.095              | 86.373               |
| 67          | C012137          | Tuwachung, Khotang    | Isthaniya bhatta  | 649                 | 27.095              | 86.373               |
| 68          | C012153          | Myagde, Tanahu        | Kalo bhatta       | 499                 | 27.995              | 84.172               |
| 69          | C012164          | Bhimad, Tanahun       | Seto bhatmaas     | 453                 | 27.976              | 84.075               |
| 70          | C012345          | Ghiring, Tanahun      | Khairo bhatmaas   | 338                 | 27.873              | 83.949               |
| 71          | C012382          | Rampur, Palpa         | Khairo bhatmaas   | 400                 | 27.853              | 83.882               |
| 72          | C012554          | Bhimad, Tanahu        | Kalo bhatmaas     | 892                 | 27.956              | 83.982               |
| 73          | C012662          | Biruwa, Syangja       | Local bhatta      | 879                 | 28.001              | 83.913               |
| 74          | C012714          | Biruwa, Syangja       | Local bhatta      | 879                 | 28.001              | 83.913               |
| 75          | C012890          | Dhanpa, Dhankuta      | Local bhatmaas    | 1530                | 27.009              | 87.310               |
| 76          | C013253          | Dhanpa, Dhankuta      | Seto bhatmaas     | 1530                | 27.009              | 87.310               |
| 77          | C013509          | Miklajung, Paachthar  | Kailo bhatmaas    | 1699                | 26.866              | 87.666               |

### *Assessment and Analysis of Agro-morphological Traits*

Agro-morphological traits were evaluated at crop development stages using established soybean descriptors (IBPGR, 1984). Eleven qualitative traits such as seedling vigour, hypocotyl colour, flower colour, stem determination, corolla colour, leaflet shape, colour and type of pubescence, the colour of the pod, seed coat pattern and colour were examined at various stages of growth. In addition, twelve quantitative traits were recorded as outlined in the descriptors: time to 50% bloom, length and width of terminal leaf, maturity duration, height of the plant at full growth, length and width of pod, seed count per pod, length and width of seed, 1000 grain weight, and yield.

Descriptive statistics, including the arithmetic mean, range, coefficient of variation, and diversity index ( $H'$ ), were computed using MS Excel 2019. Multivariate analysis was performed using OriginPro 2024 software and R Studio version 3.5. Shannon–Weaver diversity indices were utilized to evaluate

the phenotypic diversity of each qualitative and quantitative trait, with calculations performed in MS Excel (Ghimire *et al.*, 2018; Thapa *et al.*, 2022).

## Results

### *Diversity in Morphological Traits*

All the qualitative traits analyzed exhibited polymorphism, with diversity indices ( $H'$ ) varying from 0.43 to 0.98, reflecting a spectrum of diversity from medium to extremely high among the soybean landraces. The highest diversity index was observed for flower colour (0.98), followed by corolla colour (0.97), seedling vigour (0.94), leaflet shape (0.91), and stem determination (0.88). Regarding seedling vigour, 42% of the landraces exhibited vigorous growth, 40% showed medium vigour, and 18% had poor vigour. The diversity index for seed coat colour was also notably high at 0.80, with 38% of the landraces displaying a yellowish-white seed coat as detailed in table 2.

Table 2: Diversity Based on Qualitative Traits

| <i>S.N.</i> | <i>Qualitative characters</i> | <i>Shannon-weaver index</i> | <i>Descriptor's states</i>                              | <i>Numbers of landraces</i> | <i>Frequency %</i> |
|-------------|-------------------------------|-----------------------------|---------------------------------------------------------|-----------------------------|--------------------|
| 1.          | Seedling vigor                | 0.94                        | 3-Poor<br>5-Medium<br>7- Vigorous                       | 14<br>31<br>32              | 18<br>40<br>42     |
| 2.          | Hypocotyl colour              | 0.43                        | 1-Green<br>2-Purple                                     | 70<br>7                     | 90<br>10           |
| 3.          | Flower color                  | 0.98                        | 1- Purple<br>4- White                                   | 33<br>44                    | 43<br>57           |
| 4.          | Stem determination            | 0.88                        | 3- Determinate<br>5-Semi-determinate<br>9-Indeterminate | 22<br>43<br>12              | 28<br>56<br>16     |
| 5.          | Corolla color                 | 0.97                        | 3- White<br>5- Purple throat<br>7- Purple               | 33<br>24<br>20              | 43<br>31<br>26     |
| 6.          | Leaflet shape                 | 0.91                        | 3- Narrow<br>5-Intermediate<br>7-Broad                  | 12<br>37<br>28              | 16<br>48<br>36     |
| 7.          | Pubescence colour             | 0.63                        | 1- Grey<br>2- Light brown<br>3- Brown                   | 7<br>59<br>11               | 9<br>77<br>14      |
| 8.          | Pubescence type               | 0.48                        | 1-Erect<br>2-Semi-appressed<br>4- Erect                 | 35<br>48<br>1               | 41<br>56<br>1      |
| 9.          | Mature pod colour             | 0.72                        | 3- Tan<br>5- Brown<br>7- Black                          | 2<br>39<br>36               | 3<br>51<br>46      |
| 10.         | Seed coat color               | 0.80                        | 1-Yellowish<br>White                                    | 29<br>1                     | 38<br>1            |

| <i>S.N.</i> | <i>Qualitative characters</i> | <i>Shannon-weaver index</i> | <i>Descriptor's states</i>                                      | <i>Numbers of landraces</i> | <i>Frequency %</i>  |
|-------------|-------------------------------|-----------------------------|-----------------------------------------------------------------|-----------------------------|---------------------|
|             |                               |                             | 2- Yellow<br>3-Green<br>4-Buff<br>6-Imperfect black<br>8- Black | 1<br>17<br>14<br>15         | 1<br>22<br>18<br>20 |
| 11.         | Seed coat pattern             | 0.67                        | 1- Light hilum<br>2- Dark hilum<br>3- Saddle                    | 37<br>39<br>1               | 48<br>51<br>1       |

### *Diversity in Quantitative Traits*

The study revealed substantial variation in quantitative traits among the soybean landraces, as evidenced by a wide range in the coefficient of variation from 5.74% (days to maturity) to 112.49% (yield). This significant variability underscores the differences in crucial traits such as grain yield, 1000-seed weight, pod length, and plant height at maturity. The diversity within these traits was further quantified using the Shannon-Weaver index, which ranged from 0.50 to 0.99, indicating intermediate to very high diversity levels. Notably, plant height at maturity exhibited the highest diversity index (0.99), followed closely by pod width (0.95), seed length (0.89), 1000-seed weight (0.89), number of seeds per pod (0.88), and terminal leaflet length (0.88). These findings highlight the significant genetic diversity present in these traits. The remaining quantitative traits displayed intermediate to high diversity, demonstrating the rich genetic variation within the soybean landraces as detailed in table 3.

Table 3: Quantitative Traits-Based Diversity

| <i>S.N.</i> | <i>Traits</i>                | <i>Mean±SE</i> | <i>Std.</i> | <i>CV%</i> | <i>Max.</i> | <i>Min.</i> | <i>(H')</i> |
|-------------|------------------------------|----------------|-------------|------------|-------------|-------------|-------------|
| 1           | Days to 50% flowering        | 88±1.28        | 11.17       | 12.7       | 108         | 63          | 0.72        |
| 2           | Terminal leaflet length (cm) | 13.8±0.18      | 1.63        | 11.9       | 17.1        | 9.9         | 0.88        |
| 3           | Terminal leaflet width (cm)  | 7.8±0.14       | 1.24        | 15.8       | 10.6        | 4.9         | 0.57        |
| 4           | Days to 80% maturity         | 154±1.01       | 8.85        | 5.74       | 178         | 135         | 0.72        |
| 5           | Plant height at maturity(cm) | 124.2±0.03     | 33.14       | 26.7       | 252.5       | 75.60       | 0.99        |
| 6           | Pod length (cm)              | 4.4±0.13       | 1.19        | 27.3       | 11.9        | 3.34        | 0.50        |
| 7           | Pod width (mm)               | 8.8±0.12       | 1.05        | 11.9       | 11.1        | 6.61        | 0.95        |
| 8           | No seeds/Pod                 | 2.4±0.04       | 0.35        | 14.8       | 3.0         | 2.00        | 0.88        |
| 9           | Seed length (mm)             | 7.6±0.09       | 0.87        | 11.4       | 9.4         | 5.2         | 0.89        |
| 10          | Seed width (mm)              | 5.7±0.16       | 1.42        | 24.8       | 15.3        | 2.84        | 0.77        |
| 11          | 1000 grain weight (gm)       | 134±5.86       | 51.46       | 38.2       | 245.2       | 46.2        | 0.89        |
| 12          | Grain yield (ton/ha)         | 0.38±0.04      | 0.43        | 112.5      | 2.2         | 0.02        | 0.73        |

*Note:* Std.: Standard Deviation, CV: Coefficient of Variation, Min: Minimum, Max.: Maximum, H: Shannon–Weaver diversity index, SE: Standard Error

### *Cluster Analysis*

A dendrogram was generated for the 77 soybean landraces utilizing the UPGMA clustering approach, which incorporates average linkage and Euclidean

distance, to analyze 12 quantitative traits. The cluster segmentation organized the landraces into two distinct categories, resulting in the formation of two separate clusters with a distance of 111.42. Cluster 1 contained 66 soybean landraces (85.71%), while Cluster 2 included 11 landraces (14.29%). Landraces in Cluster 1 exhibited elevated values for maturity duration, 1000-grain weight, height of the matured plant, days to flowering, total leaf length (TLL), and grain yield. Within Cluster 1, specific landraces demonstrated superior performance in various traits: C02834 and C02831 excelled in grain yield; C010877 and C011150 had higher 1000-grain weight; C011174 and C011867 flowered earlier. Cluster 2 consists of landraces with higher values for plant height, flowering duration, 1000-grain weight, total leaf length (TLL), and seeds per pod. Within Cluster 2, landraces like C0715 and C02839 were superior in terms of maturity duration; landraces like C011165 and C02829 excelled in the number of seeds count per pod and landrace C0741 was superior in plant height. For key economic and physiological traits like grain yield, 1000-grain weight, early flowering days, and early maturity days, Cluster 1 can be selected. However, Cluster 2 is also economically significant due to its higher number of seeds per pod and can be regarded as suitable for selection to boost seed production.

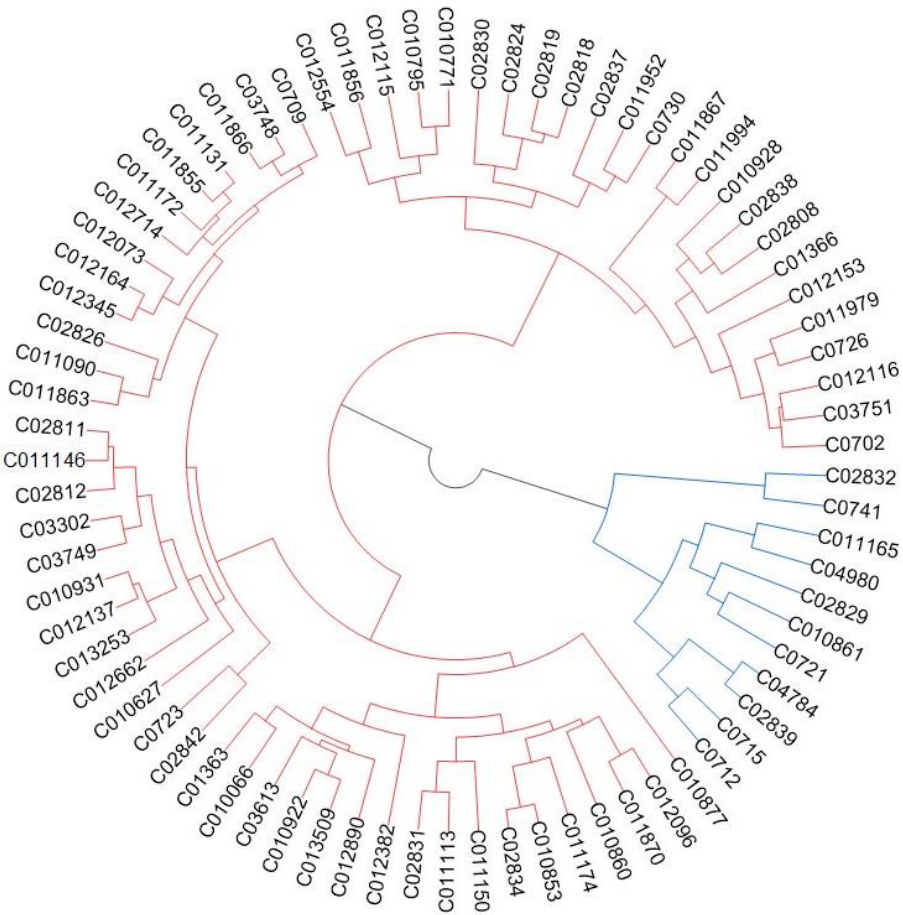


Figure 2: UPGMA Clustering of 77 Soybean Landraces Based on Twelve Quantitative Trait

Table 4: Mean Value of Quantitative Traits in Each Cluster

| <i>Variables</i>          | <i>Cluster-1</i> | <i>Cluster-2</i> |
|---------------------------|------------------|------------------|
| Number of landraces       | 66               | 11               |
| Days to 50% flowering     | 87.45            | 91.81            |
| Terminal leaf length (cm) | 14.18            | 11.40            |
| Terminal leaf width (cm)  | 8.41             | 6.13             |
| Days to maturity          | 154.54           | 152              |
| Plant height (cm)         | 113.72           | 187.26           |
| Pod length (cm)           | 4.35             | 4.68             |
| Pod width (mm)            | 9.02             | 7.61             |
| Number of seeds/pods      | 2.36             | 2.56             |
| Seed length (mm)          | 7.80             | 6.85             |
| Seed width (mm)           | 5.73             | 5.71             |
| 1000 grain wt. (gm)       | 146.71           | 63.25            |
| Yield (t/ha)              | 0.41             | 0.200            |

*Principal Component Analysis*

In this analysis, the initial four components play a crucial role in capturing the variation patterns among landraces, and the traits linked to these components are more effective in distinguishing accessions. The initial four components, each with an eigenvalue exceeding 1, explained 86.07% of the total variability (Figure 2). First, second, third and fourth components accounted for 37.87%, 21.20%, 18.2% and 8.8%, respectively. The total variance of the first component indicates that terminal leaf width, 1000 grain wt., terminal leaf length, pod width, plant height, days to flowering and seed length were the most contributed variables (Figure 3). The second principal component, which captures 21.20% of the overall variability, was predominantly affected by factors such as maturity duration, seed count per pod, seed length, and pod width (Figure 4).

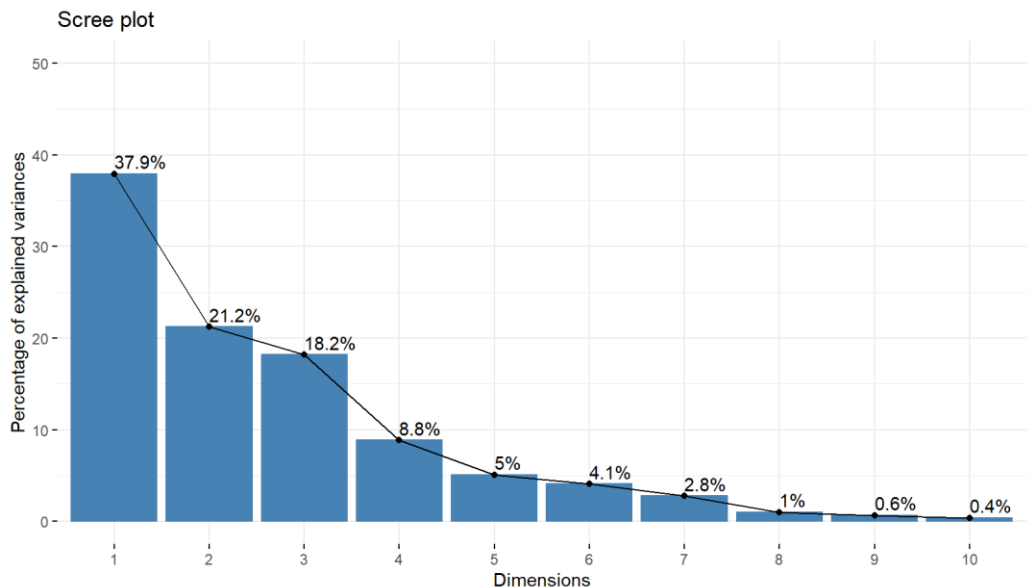


Figure 3: Influence of Various Principal Components

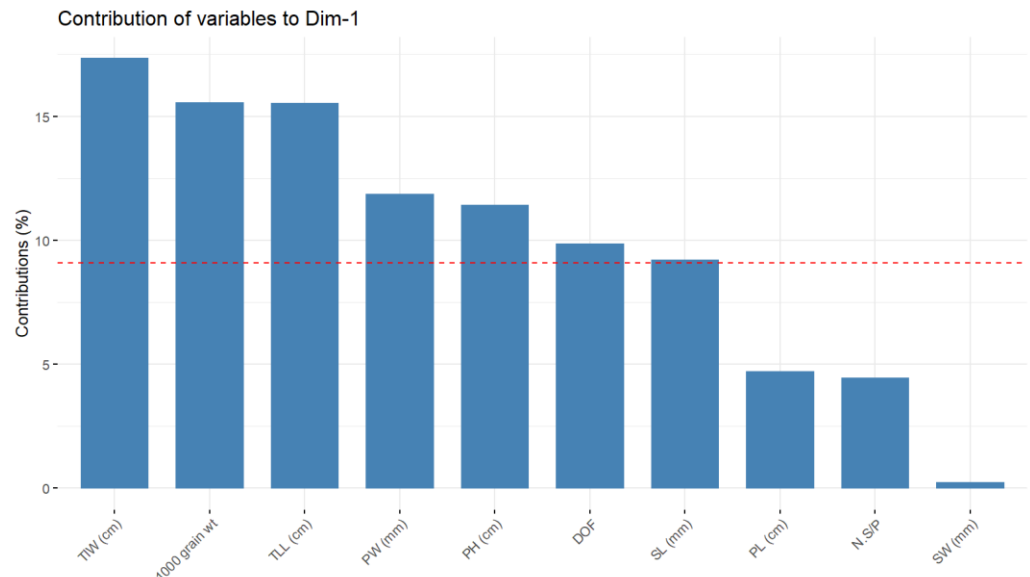


Figure 4: Influence of Different Traits on the First Principal Component

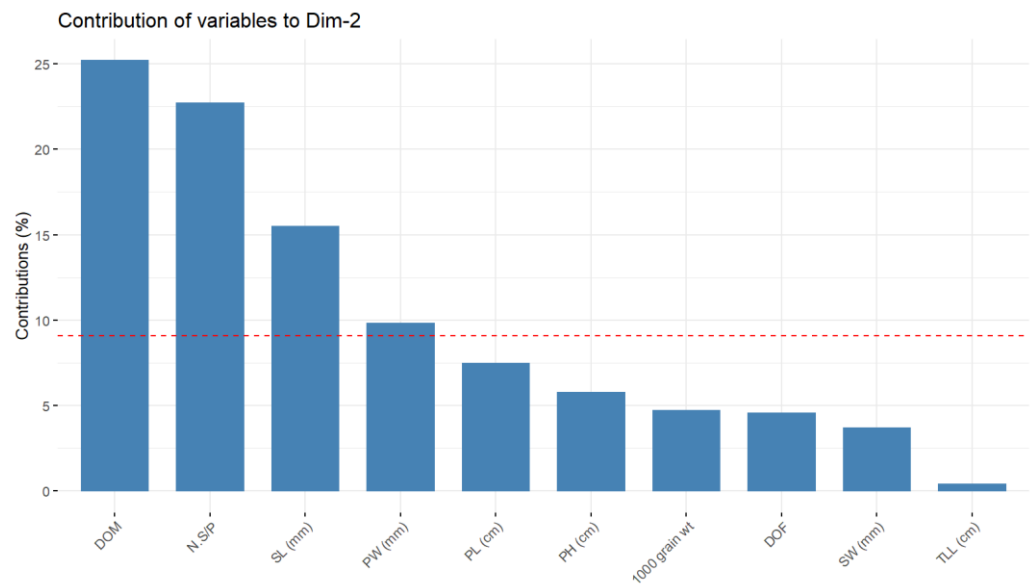


Figure 5: Influence of different traits on the Second Principal Component

The PC1 has high loadings on 1000 grain wt., seed length, pod width, terminal leaf length and width. Hence PC1 is strongly positively associated with these variables. The projection of variable plant height on PC1 shows large negative values. Likewise, PC2 has high loadings on pod length, maturity and flowering duration, seed count per pod and seed width. Conversely, grain yield has negative correlations with PC2 (Figure 5). Seed width and 1000-grain weight coincide, indicating a perfectly positive relationship between the two traits. Days to flowering formed a large angle with grain yield suggesting that they are negatively correlated. Likewise, seed count per pod is inversely related to seed width and 1000 grain weight.

Displaying the accessions along the first and second principal components indicated that most of the accessions were in the centre (Figure 6). Some landraces that were distantly plotted from the centre were C02834, C011113, C011174 and C02831. These distantly located accessions in the biplot had extreme value at one trait i.e. grain yield.

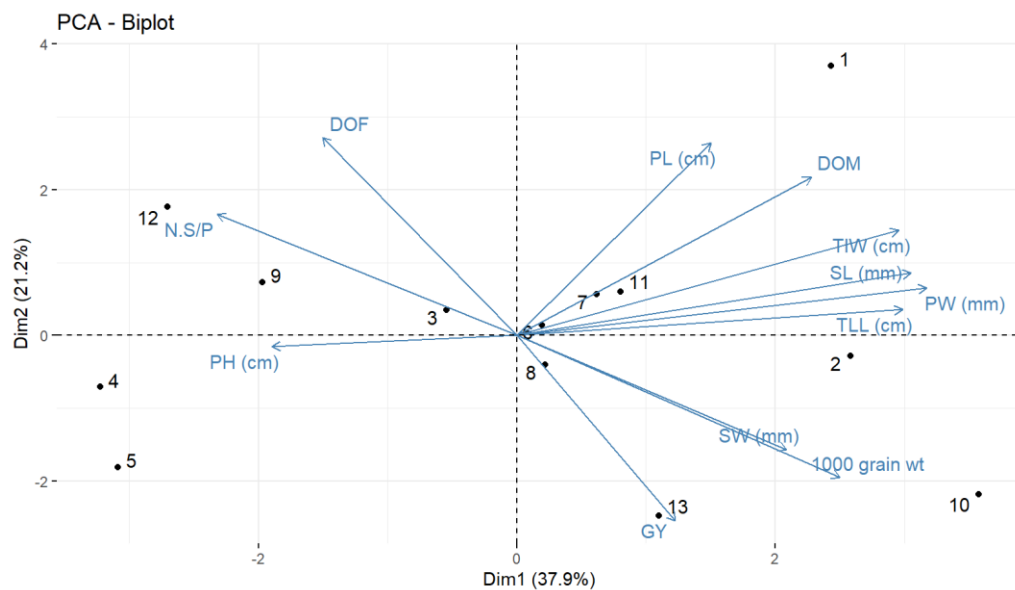


Figure 6: PCA Biplot of Soybean Landraces for 11 Quantitative Traits

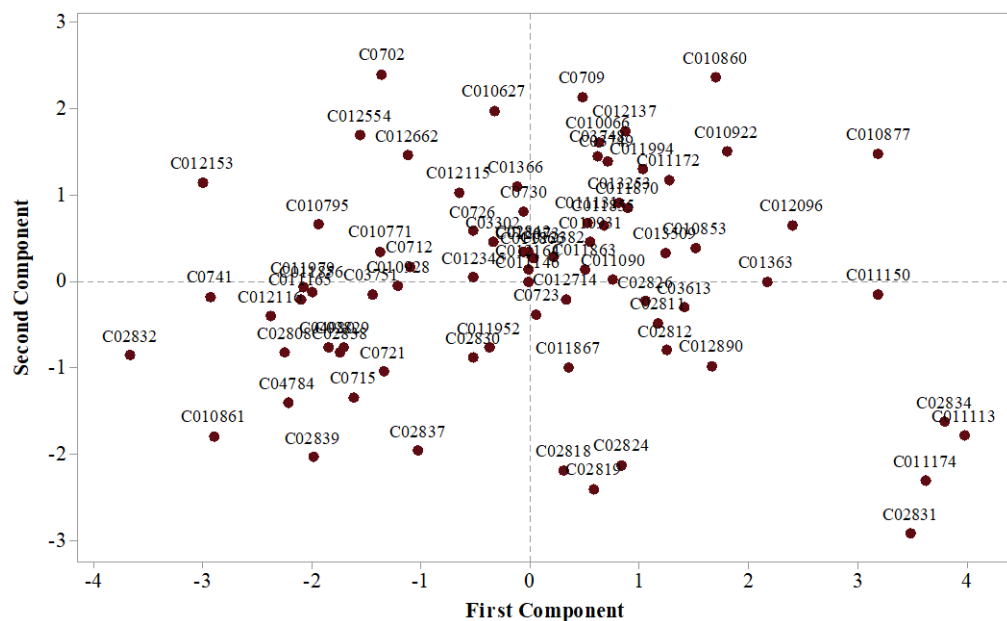


Figure 6: Soybean landraces categorized by the 1<sup>st</sup> and 2<sup>nd</sup> principal components

## Discussion

As indicated by the present study, all morphological characters under study are found to be polymorphic in Nepalese soybean landraces. Knowledge of

the variability for each trait within the collected germplasm highlights the potential for improving these traits through selective breeding (Naik *et al.*, 2016). The identified variability can be further assessed for pest resistance, stress tolerance, and yield potential to enhance its utility (Chavez *et al.*, 2017). The characterized landraces had diverse types of seedling vigour. Among them, 42% had the vigorous type of seedlings which have a greater ability of translocation of reserve materials and ultimately help to increase yield (Meneguzzo *et al.*, 2021). Seed coat colour is a trait in soybeans, which has evolved from multi-coloured during the transition from primitive to domesticated forms (Liu *et al.*, 2021). Seed coat colour, a characteristic often seen in domestication, has shifted from black to various hues including yellow, green, brown, and bicolour as soybeans have been domesticated from their wild origins to cultivated forms. (Liu *et al.*, 2021). In our study, thirty-eight per cent of the landraces had the yellowish-white type of seed coat colour. Shurtleff and Aoyagi (2014) reported that the commonly found soybean seed coat colour is yellow. Nepalese soybeans had diverse types of leaflet shapes. Sixteen per cent, 48% and 36% of landraces had narrow, intermediate and broad types of leaflets respectively. Narrow leaflets are linked to an increased number of seeds per pod in *G. max* (Chen *et al.*, 2004). You *et al.* (1995) examined 72 cultivated soybeans from three countries to assess the impact of the shape of the leaflet on seed components. Their study concluded that leaflet size did not significantly affect crop yield or the pods per plant, but significant differences were observed in seed dimension and the seed count per pod. Bilyeu *et al.* (2016) emphasized that leaf morphology, growth pattern, pod colour, and types of hairiness are essential morphological traits in soybean research.

The landraces showed significant variability for the 12 quantitative traits. A large coefficient of variation was observed in important traits like grain yield (112.49%), 1000 grain weight (38.18%), pod length (27.25%) and plant height at maturity (26.68%) indicating significant variation among the landraces for these characteristics. Malik *et al.* (2006) recorded high coefficients of variation in traits like leaf surface area, pod count per plant, branch count per plant, thousand seed weight, and plant grain output. He documented plant heights varying between 22 and 93 cm. In our study, plant height at maturity ranged from 75.60 to 252.48 cm. Soybean landraces with higher plant height can be used for fodder purposes. In the USA and China, soybean plant with high foliage is used for cattle which are very palatable, have a high nutritive value and good digestibility (Mukosi, 2020). Foliage soybeans can be used for making high-quality hay which can be harvested from the flowering stage to near maturity (Sterna *et al.*, 2023). Our study showed that 1000 grain wt. ranged from 48-245 gm. Maximum 1000 grain wt. i.e. 245 gm was found in landrace C010877. Seed size is a key attribute with a direct impact on soybean yield (Hina *et al.*, 2020). Perry (1981) reported a significant relationship between seed size, seedling development, and the availability of dietary reserves in seeds. An increase in seed size is expected to positively impact seedling growth. Under optimal germination and growth conditions, larger seeds will yield bigger seedlings than smaller seeds, resulting in higher biomass production. Under favourable germination and growth conditions, large seeds will produce larger seedlings compared to small seeds, leading to increased crop production.

Among the tested landraces, CO2819 has shorter days to maturity (135 days) which can be utilized for developing early matured varieties. Chavez *et al.*

(2017) documented that the duration to reach 50% maturity ranged from 91 to 109 days. Likewise, landraces CO2831 and CO2834 had grain yields of 2.20 and 2.07 ton/ha respectively so these landraces can be further screened for the development of high-yielding varieties. These findings are consistent with the observations of Rasaily *et al.* (1986), who noted considerable genotypic variability in seed yield through their experiments. Funnah and Mak (1978) also carried out research trials and discovered that certain varieties produced yields exceeding 2 tons/ha.

Characterizing and grouping landraces depend on their phenological and genetic similarities to support identifying and selecting optimal parents for hybridization (Kumar *et al.*, 2022). Thus, using multivariate analysis offers breeders valuable insights, enabling them to identify and select the most promising landraces for early-stage breeding and additional evaluation (Thapa *et al.*, 2022). Derai *et al.* (2020) reported that the clustering of 40 soybean accessions, based on seven morphological traits, categorized them into five primary groups at a genetic dissimilarity of 202.63. Similarly, Khadka *et al.* (2020) constructed the dendrogram of sixteen genotypes of soybean using fifteen morphological traits in four major clusters. The overall PCA analysis highlighted variations in the quantitative descriptors across the individual components. In breeding, traits that contribute significantly to the maximum variability are particularly valuable. For these landraces, traits such as length and width of apical leaf, thousand seed weight, maturity duration, seed count per pod, plant height at maturity, and dimensions of pods and seeds are crucial for genetic diversity studies. Descriptors with high loading values are anticipated to exert a more significant influence on overall diversity (Chavez *et al.*, 2017). The most influential variables, characterized by the most prominent descriptors within the groups, are determined by the length of their vectors (Dossa *et al.*, 2011).

The study has some limitations, such as restricted geographic coverage and potential environmental variability affecting trait expression. Future research should aim to include more diverse regions, conduct genetic analysis, and validate trait correlations under varying conditions. Additionally, evaluating stress tolerance, nutritional quality, and adaptability over time could further enhance the value of Nepalese soybean landraces for breeding and sustainable cultivation.

## Conclusion

The soybean landraces studied exhibited considerable variability in both qualitative and quantitative traits, underscoring their significant genetic diversity and potential for various applications. This observed diversity not only highlights the adaptability of these landraces to diverse agro-climatic conditions but also provides valuable genetic resources for soybean breeding programs. Traits such as days to maturity and yield demonstrated remarkable variation, emphasizing the scope for selecting and enhancing desirable characteristics. Preliminary evaluations identified CO2819 (Seto Bhatmaas) from Ramechhap, CO2831 (Kaili Bhatmaas) from Kavrepalanchok, and CO2834 (Seto Bhatmaas) from Dolakha as particularly promising landraces. These accessions exhibited superior performance in crucial traits, making them potential for breeding high-yielding and stress-tolerant soybean varieties. However, to fully exploit their potential, further research is necessary. Multi-environment trials are essential to

evaluate their stability and adaptability under different ecological conditions. The promising landraces identified in this study should be prioritized for detailed genetic and environmental evaluations to ensure their effective utilization in future soybean breeding and cultivation strategies.

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## Authors' Declarations and Essential Ethical Compliances

### *Authors' Contributions (in accordance with ICMJE criteria for authorship)*

| <i>Contribution</i>                             | <i>Author<br/>1</i> | <i>Author<br/>2</i> | <i>Author<br/>3</i> | <i>Author<br/>4</i> | <i>Author<br/>5</i> |
|-------------------------------------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| Conceived and designed the research or analysis | Yes                 | Yes                 | No                  | No                  | No                  |
| Collected the data                              | No                  | No                  | No                  | No                  | No                  |
| Contributed to data analysis and interpretation | Yes                 | No                  | No                  | No                  | No                  |
| Wrote the article/paper                         | Yes                 | Yes                 | No                  | No                  | No                  |
| Critical revision of the article/paper          | No                  | No                  | No                  | Yes                 | No                  |
| Editing of the article/paper                    | No                  | No                  | Yes                 | No                  | No                  |
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| Funding Acquisition                             | 50                  | 20                  | 10                  | 10                  | 10                  |
| Overall Contribution Proportion (%)             | 50                  | 20                  | 10                  | 10                  | 10                  |

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### *Research involving human bodies or organs or tissues (Helsinki Declaration)*

The author(s) solemnly declare(s) that this research has not involved any human subject (body or organs) for experimentation. It was not a clinical research. The contexts of human population/participation were only indirectly covered through literature review. Therefore, an Ethical Clearance (from a Committee or Authority) or ethical obligation of Helsinki Declaration does not apply in cases of this study or written work.

### *Research involving animals (ARRIVE Checklist)*

The author(s) solemnly declare(s) that this research has not involved any animal subject (body or organs) for experimentation. The research was not based on laboratory experiment involving any kind animal. The contexts of animals were only indirectly covered through literature review. Therefore, an Ethical Clearance (from a Committee or Authority) or ethical obligation of ARRIVE does not apply in cases of this study or written work.

### *Research on Indigenous Peoples and/or Traditional Knowledge*

The author(s) solemnly declare(s) that this research has not involved Indigenous Peoples as participants or respondents. The contexts of Indigenous Peoples or Indigenous Knowledge were only indirectly covered through literature review. Therefore, an Ethical Clearance (from a Committee or Authority) or prior informed consent (PIC) of the respondents or Self-Declaration in this regard does not apply in cases of this study or written work.

### *Research involving Plants*

The author(s) solemnly declare(s) that this research has involved the plants for experiment and field studies. Some contexts of plants are also indirectly covered

through literature review. Thus, during this research the author(s) obeyed the principles of the Convention on Biological Diversity and the Convention on the Trade in Endangered Species of Wild Fauna and Flora.

*Research Involving Local Community Participants (Non-Indigenous) or Children*

The author(s) solemnly declare(s) that this research has not directly involved any local community participants or respondents belonging to non-Indigenous peoples. Neither this study involved any child in any form directly. The contexts of different humans, people, populations, men/women/children and ethnic people were only indirectly covered through literature review. Therefore, an Ethical Clearance (from a Committee or Authority) or prior informed consent (PIC) of the respondents or Self-Declaration in this regard does not apply in cases of this study or written work.

*(Optional) PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses)*

The author(s) has/have NOT complied with PRISMA standards. It is not relevant in case of this study or written work.

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